LIVER BROTH: A MEDIUM FOR THE DETERMINATION OF GAS-FORMING BACTERIA IN WATER.*

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Various observers have noted that when dextrose broth is used for the bacterial examination of water it occasionally gives a positive test for *B. coli* in one and sometimes in two dilutions higher than does the lactose bile medium. This is particularly the case when waters of fairly good sanitary quality are tested. In tests on sewage and contaminated waters, however, the lactose bile has been proven to give positive results in higher dilutions than any other medium, the gas production being more vigorous and anomalies caused by the overgrowth of other forms very rare.

After numerous experiments, it has been found that the lactose bile medium is slightly inhibitive to *B. coli*, especially in attenuated form, so that any positive tests with this medium indicate recent and fresh contamination.

Dextrose broth, containing no inhibitive substance, favors to some extent the attenuated forms, but often overgrowth by other bacteria more numerous than *B. coli* renders the test either doubtful or negative.

This feature of dextrose broth has been overcome by the use of a broth made from fresh beef liver. The latter medium gives all the gas-producing bacteria present, attenuated or otherwise, including those forms which ferment dextrose but not lactose. It therefore has every advantage over the use of dextrose broth for general gas production and preliminary rejuvenation.

If one sugar medium alone is used in the sanitary examination of water, the lactose bile should be employed, as it gives relative results which are entirely comparable with each other on the actual sanitary condition of the waters under examination. If a further study of all gas-forming bacteria including attenuated forms is desirable, then liver broth should be employed in preference to the

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usual dextrose broth, as it gives a larger amount of attenuated forms, has better rejuvenating power, and gives fewer anomalies and greater and more rapid gas production. This medium has been in use at Mt. Prospect Laboratory for over two years and has proved its value in all of these respects.

The composition of the medium is as follows:

LIVER BROTH.

Beef liver	500.0 gms.
Peptone (Witte's)	10.0 "
Dextrose	10.0 "
Potassium Phosphate (K_2HPO_4)	1.0 "
Water	1,000.0 "

1. Chop 500 gms. of beef liver into small pieces and add 1,000 c.c. of distilled water. Weigh the infusion and container.

2. Boil slowly for two hours in a double boiler, starting cold, and stirring it occasionally.

3. Make up the loss in weight by evaporation and strain through a wire strainer.

4. To the filtrate add 10 gms. of peptone, 10 gms. of dextrose, and one gm. of potassium phosphate. Weigh the infusion and container.

5. After warming this mixture in a double boiler and stirring it for a few minutes to dissolve the ingredients, titrate with N/20 sodium hydrate, using phenolphthalein as an indicator, and neutralize with normal sodium hydrate.

6. Boil vigorously for 30 minutes in a double boiler, and five minutes over a free flame with constant stirring to prevent the caramelization of the dextrose.

7. Make up any loss in weight by evaporation and filter through cotton flannel and filter paper.

8. Tube and sterilize in an autoclave for 15 minutes at 120° C. (15 lbs.).

Other valuable liver media (for use in the identification of *B. sporogenes* and other species) are prepared as given below:

LIVER GELATIN.

1. Proceed as in steps 1, 2, 3 in preparing liver broth.

2. Cool the filtrate to 50° C. Add 10 per cent of sheet gelatin and stir a few minutes until dissolved.

3. Add one per cent of peptone, one per cent of dextrose, and one-tenth (0.1) per cent of potassium phosphate.

4. Stir until the ingredients are dissolved, keeping the temperature below 50° C., and then proceed as in steps 5, 6, 7, 8 in preparing liver broth.

LIVER AGAR.

1. Chop 500 gms. of beef liver into small pieces, add 500 c.c. of distilled water, and boil slowly for two hours, stirring occasionally.

2. Add five gms. of agar (dried at 105° C. for 30 minutes) to 500 c.c. of distilled water and digest for 30 minutes in an autoclave at 120° C. (15 lbs.).

3. After making up the loss by evaporation, strain the liver infusion through a wire strainer, add 500 c.c. of the filtrate to the agar solution and proceed as in steps 4, 5, 6, 7, 8 in preparing liver broth.

It is very important to note that liver broth should not be exposed to the high temperature attained in the autoclave any longer than 15 minutes, as prolonged heating above the boiling point causes caramelization of the carbohydrates, rendering the medium less delicate for bacterial development. For the rejuvenation of species, especially *B. sporogenes*, the addition of very small pieces of liver tissue which have been sterilized in Petri dishes in the autoclave for 15 minutes improves the rejuvenating properties of the medium. They should be added to the tubes after sterilization.

Bacterial growth being very rapid in this medium, preliminary rejuvenation at 37° C. should be concluded between six and 12 hours.

It was determined by the chemical analyses of several different lots of liver broth, before and after sterilization, that the beef liver as purchased in the market and occurring in the sterilized medium usually contains about one-tenth to two-tenths of one per cent of glycogen, to which it probably owes its principal rejuvenating properties. The authors are indebted to Mr. John E. Dowd, of the Mt. Prospect Laboratory, for numerous glycogen determinations.

The following table will show the comparative results obtained by planting samples of water directly into lactose bile, dextrose broth, and liver broth, as well as by preliminary rejuvenation in nutrient broth and liver broth, and then planting into lactose bile. In four cases all tubes showing gas formation with liver broth in higher dilution than with lactose bile were transplanted into lactose bile to determine whether the attenuated colon bacillus was among the gas-formers present.

The figures on p. 292 show that in waters where the good sanitary quality is in part due to storage, as in the city tap samples and No. 4 Pond, little or no gas was obtained in the lactose bile; considerable gas was often found in the dextrose broth, while in liver broth the growth was more vigorous, as is shown by the gas production in shorter periods, and in larger amounts as well as in higher dilutions.

SAMPLE	BACTERIA PER C.C. AT 20° C.	HRS.	LACTOSE BILE: PER CENT GAS						DEXTROSE BROTH: PER CENT GAS						LIVER BROTH: PER CENT GAS						FROM NUTRIENT BROTH INTO LACTOSE BILE: PER CENT GAS						FROM LIVER BROTH INTO LACTOSE BILE: PER CENT GAS					
			10 c.c	1 c.c	.1 c.c	.01 c.c	.001 c.c.	.0001 c.c.	10 c.c	1 c.c	.1 c.c	.01 c.c	.001 c.c.	.0001 c.c.	10 c.c	1 c.c	.1 c.c	.01 c.c	.001 c.c.	.0001 c.c.	10 c.c	1 c.c.	.1 c.c.	.01 c.c.	.001 c.c.	.0001 c.c.	10 c.c.	1 c.c.	.1 c.c.	.01 c.c.	.001 c.c.	.0001 c.c.
1. Deep Well..	10	24	0	0	0	0	..	...	0	0	0	0	..	...	0	0	0	0	..	...	0	0	0	0	...	....	0	0	0	0	...	....
		48	0	0	0	0	..	...	0	0	0	0	..	...	0	0	0	0	..	...	0	0	0	0	...	....	0	0	0	0	...	....
		72	0	0	0	0	..	...	0	0	0	0	..	...	0	0	0	0	..	...	0	0	0	0	...	....	0	0	0	0	...	....
2. N.Y. City Tap ...	210	24	0	0	0	0	..	...	2	13	3	0	..	...	80	37	10	0	..	...	15	0	0	0	...	....	85	22	0	0	...	....
		48	0	0	0	0	..	...	13	32	18	0	..	...	80	45	21	0	..	...	18	0	0	0	...	....	85	28	85	0	...	....
		72	0	0	0	0	..	...	24	30	20	0	..	...	83	50	28	0	..	...	21	0	0	0	...	....	85	35	85	0	...	....
3. N.Y. City Tap ...	175	24	0	0	0	0	..	...	0	0	0	0	..	...	12	13	12	0	..	...	50	18	0	0	...	....	35	12	0	0	...	....
		48	0	0	0	0	..	...	20	0	0	0	..	...	33	45	30	0	..	...	53	38	40	0	...	....	65	20	8	0	...	....
		72	17	0	0	0	..	...	20	0	0	0	..	...	37	47	38	0	..	...	53	45	52	0	...	....	75	26	23	0	...	....
4. Pond.....	780	24	0	0	0	0	..	...	3	8	5	0	..	...	20	24	9	18	..	...	8	0	0	0	...	....	38	10	0	0	...	....
		48	0	0	0	0	..	...	10	20	11	0	..	...	38	35	85	32	..	...	33	0	0	0	...	....	42	18	0	0	...	....
		72	0	0	0	0	..	...	12	26	12	0	..	...	38	38	90	35	..	...	42	0	0	0	...	....	47	21	0	0	...	....
5. Pond.....	175	24	0	0	0	0	..	...	13	12	16	0	..	...	17	13	18	2	..	...	35	0	0	0	...	....	42	38	3	0	...	....
		48	28	23	0	0	..	...	38	28	32	0	..	...	40	15	77	26	..	...	48	35	0	0	...	....	56	46	10	0	...	....
		72	34	45	0	0	..	...	45	40	35	0	..	...	45	13	83	33	..	...	50	40	0	0	...	....	67	62	13	0	...	....
6. Polluted Brook...	15,000	24	42	55	22	0	0	0	14	18	30	0	0	0	45	28	40	25	15	0	..	..	..	..	...	....	..	..	..	..	...	....
		48	56	75	47	43	0	0	35	32	56	57	10	0	55	33	50	35	32	0	..	..	..	..	...	....	..	..	..	..	...	....
		72	58	78	50	45	0	0	38	35	55	68	16	0	55	30	52	35	32	0	..	..	..	..	...	....	..	..	..	..	...	....

In No. 5 Pond, where the pollution is more recent, the lactose bile shows the extent of such pollution, while in the dextrose broth and liver broth the distinction is not so marked. The same is true with the more highly polluted water shown in No. 6.

The figures on attenuated coli obtained by rejuvenating before planting into lactose bile show that preliminary rejuvenation in liver broth gives higher results than nutrient broth.

In a long series of experiments with various kinds of waters, in which all tubes showing gas formation in liver broth in higher dilution than in lactose bile were transplanted into lactose bile, about 65 per cent were positive after this rejuvenation.

It was observed that gas production in the liver broth tubes was usually well advanced within 24 hours, thus giving a prompt indication of the presence of gas-forming bacteria.

The following experiment will give a fairly good idea of the value of liver broth as a rejuvenating medium for attenuated *B. coli*.

A sample of surface water containing 300 bacteria per c.c. and no gas-formers of any kind was inoculated with a pure culture of *B. coli*, and kept in a stoppered bottle in the refrigerator for about four months.

At various times during this period this mixture was planted into lactose bile, dextrose broth, and liver broth.

Gas formation was obtained in dilutions varying from .0001 c.c. to 10 c.c., until finally gas formation was negative with 10 c.c. of the water in lactose bile and dextrose broth, even after rejuvenation in nutrient broth. The liver broth continued to yield vigorous gas formation, within 24 hours after inoculation with one c.c. of the water, several weeks longer. All tubes showing gas with liver broth were transplanted into lactose bile, as before, and positive results obtained.

The value of the use of liver broth in a study of the gas-forming bacteria in water, in the presumptive evidence of their presence, and as a preliminary step in their isolation as compared with other media now in use, has been many times demonstrated in this laboratory. In work on milk, sewage, or feces it has been found to be of great assistance in isolating the various species of bacteria of intestinal origin.

Except in the case of spore-forming bacteria, transplanting from liver broth should usually be done between six and 12 hours after inoculation and incubation at 37° C., as development is generally so rapid that after that period the excessive amount of toxic products often causes considerable attenuation or inhibition.

During the course of our work on various species, it was observed that *B. sporogenes*, in addition to gas formation, gave a very offensive cheesy odor in liver broth, while other forms gave only a very slight odor.

This feature is a very good indication of its presence, and furnishes a simple means for its identification.

CONCLUSIONS.

The determination of gas-forming bacteria in water by means of lactose bile gives results which represent the relative degree of contamination of dangerous or recent origin. While of especial value in judging the degrees of pollution present, it does not show the presence of gas-formers other than the colon group and does not often indicate *B. coli* in an attenuated state.

For those observers who desire to determine the presence of all gas-formers, including *B. coli*, to the highest accuracy, the use of liver broth is recommended.

In the examination of water, dilutions of 0.1, 1.0, and 10 cubic centimeters of the sample may be inoculated into lactose bile and another series into liver broth.

Positive tests in the lactose bile indicate the degree of recent pollution with the colon group.

Gas formation in the liver broth indicates the degree of contamination with gas-forming bacteria both attenuated and vigorous.

Any difference in the results obtained in lactose bile compared with those found by transplanting within 12 hours from the liver broth into a second set of lactose bile tubes gives a fairly accurate idea of the amount of attenuated *B. coli* present. Negative results in both sets of bile tubes and positive results in the liver broth usually show that the gas-producing bacteria present are not of the colon group.